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**Agricultural landscapes and effects of pesticides in tropical highly
biodiverse streams of the Ecuadorian Choco.**

**Disertación previa a la obtención del título de
Licenciado en Ciencias Biológicas**

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Certifico que la disertación de Licenciatura en Ciencias Biológicas del candidato Andrés Morabowen Mantilla ha sido concluida de conformidad con las normas establecidas; por lo tanto, puede ser presentada para la calificación correspondiente.

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El presente estudio y la literatura citada se encuentran redactados bajo el formato de la revista científica **FRESHWATER BIOLOGY**.

1. RESUMEN

Las consecuencias a nivel de ecosistema de los usos de suelo agrícolas en Bosques tropicales del Chocó no están completamente estudiadas. Existe un conflicto fuerte entre la conservación de ecosistemas con alta biodiversidad y la explotación de áreas productivas económicamente importantes. Las prácticas agrícolas actuales involucran la deforestación completa del área de cultivo, lo que cause múltiples efectos en los ecosistemas de ríos. Para dilucidar los efectos sobre ríos tropicales del cambio en el uso de suelo, estudiamos ríos con tres tipos de usos: 1) Bosques húmedos montanos prístinos, 2) granjas orgánicas con parches de bosque y 3) monocultivos de Palmito (*Bactris gasipaes*) con uso intensivo de herbicidas (Glifosato) e insecticidas (Palmarol/Endosulfan). Se estudiaron tres ríos por cada tipo de uso de suelo. Se tomaron muestras cualitativas y cuantitativas de macroinvertebrados, se realizaron medidas cuantitativas de la cantidad de perifiton, se midió el sedimento y las variables físico químicas de cada río. Nuestros resultados muestran una relación directa entre la pérdida de algunos grupos de macroinvertebrados (e.g. *Anacroneuria*, *Hyallela* y *Nectopsyche*) y el tipo de uso de suelo. Además, encontramos que las pérdidas de diversidad de ríos que drenan de granjas orgánicas son casi nulas a nivel de macroinvertebrados y biomasa de perifiton. También encontramos que la abundancia relativa de macroinvertebrados es completamente diferente en monocultivos que en los otros dos tipos de uso de suelo. Por todas estas razones, creemos que las comunidades de los ríos que drenan el área de Mashpi están siendo transformadas debido a disturbios causados por humanos y campañas de concientización deberían ser realizadas.

Palabras clave: biodiversidad, conservación, Chocó, ecosistemas acuáticos, hotspot.

2. ABSTRACT

The ecosystem level consequences of agricultural land use in Choco tropical forests have not been fully studied. There is conflict between the conservation of highly diverse ecosystems and the use of economically important production areas. Current agricultural practices involve complete deforestation of the area, with multiple effects on stream ecosystems. To address the issue of land-use change in tropical rivers of Ecuador, we studied streams draining three different land use types in the Mashpi River drainage: 1) Pristine montane cloud forest, 2) organic farms that included forest patches, and 3) Palmito (*Bactris gasipaes*) production land with extensive use of the insecticide Endosulfan and the herbicide Glyphosate. We studied three streams in each land use type. We sampled macroinvertebrates (quantitative and qualitative samples), periphyton (quantitatively) and measured sediments and physico-chemical variables. There was a direct relationship between the decline of certain macroinvertebrate groups (e.g. *Anacroneuria*, *Hyalleya* and *Nectopsyche*) and the type of land-use. Furthermore, we found the loss of diversity in streams draining organic farms were negligible concerning macroinvertebrates and periphyton. The relative abundance of macroinvertebrates was completely different in palmito monoculture farmlands than in the other two types of land use. The stream communities of the Mashpi drainage area have been transformed due to human agricultural disturbances and awareness campaigns are necessary.

Keywords: aquatic ecosystems aquatic ecosystems, biodiversity, Choco, conservation, hotspot.

3. INTRODUCTION

The Ecuadorian Choco has a dense network of streams draining the northern Andes, to the Pacific Ocean. These streams sustain a colossal amount of different organisms. The Choco is classified as a biodiversity hot spot (Myers *et al.* 2000) and is threatened by human disturbance (Chen *et al.* 2004, Townsend *et al.* 2008, Sundbäck *et al.* 2010). Several studies have shown that different types of anthropogenic disturbances produce different effects on the biodiversity rivers support in tropical regions (Aratrakorn *et al.* 2006; Fitzherbert *et al.* 2008; Wanger *et al.* 2010). The main human related impacts to rivers and streams in this region are extensive deforestation of riverbanks performed by logging companies, conversion of forest to oil palm production accompanied by chemical river pollution, overexploitation of fisheries and channel modifications like dams. Benthic communities in stream and river networks are very effective indicators of the amount of stress these systems are experiencing. These benthic communities are very useful to evaluate environmental conditions in lotic systems because of the variety of functional roles in the ecosystem and different susceptibilities to environmental perturbations (Liess y Von der Ohe 2005; Dodds 2002).

In recent decades, the clearing of forests to make space for agriculture is the most common disturbance impacting rivers in the neotropical region (Laurance 1999), and continues to destroy millions of hectares annually (Achard *et al.* 2002; Lorion *et al.* 2009). This has been especially true in recent decades in impoverished tropical regions (Laurance 1999). Deforestation can degrade stream habitats by influencing runoff regimes and evapotranspiration patterns (Iwata *et al.* 2003b). In addition, deforestation can change water temperature regimes and alter the abundance and diversity of food resources (Henry *et al.* 1994; Benstead *et al.* 2003; Bojsen and Jacobsen. 2003; Benstead and Pringle 2004). This can lead to significant changes in benthic community structure and cause declines in

macroinvertebrate diversity (Benstead *et al.* 2003; Bojsen and Jacobsen 2003; Iwata *et al.* 2003a; Dudgeon *et al.* 2006; Wantzen and Wagner 2006).

A major problem that monoculture agricultural practices cause in ecosystems, especially in running waters, is the extensive use of pesticides that are washed into bodies of water. Glyphosate (N-(phosphonomethyl) glycine) is the most widely used herbicide in the Neotropics and worldwide (Magbauna *et al.* 2016). Pesticides have direct lethal influence on target and non-target species (Mayer *et al.* 1986), as well as indirect non-lethal effects transmitted through food webs (Relyea and Hoverman 2008; Yadav *et al.* 2015). The non-lethal effects of pesticides can be very hard to pinpoint and require long-term studies that reveal behavioral and physiological effects on vertebrates and invertebrates but can be viewed on changes in ecosystem structure and function.

There is increasing evidence that deforestation caused by conversion to agriculture has many destructive impacts on benthic communities of streams but the effects on tropical stream ecosystems are still in need of further research (Lorion and Kennedy 2009). There is little evidence to support the conservation of intact riparian forests to maintain diversity in tropical stream communities (Iwata *et al.* 2003; Tomanova *et al.* 2006; Jackson and Sweeney 1995; Dudgeon 2000). This study aims to increase knowledge about the effects of land-use changes on aquatic communities of highly biodiverse Neotropical forests. We studied the highly biodiverse aquatic communities of the Mashpi watershed in the Ecuadorian Choco biodiversity hotspot region. This area has a recent history of deforestation for the introduction of hearts of palm (*Bactris gasipaes*) monoculture. The Mashpi drainage is an ideal place to test the effects of different land uses on aquatic biodiversity because it is a mosaic of protected forests, organic agroforestry farms and palmito (*Bactris gasipaes*) monoculture. This heterogeneity allowed us to determine differences between the benthic communities in

streams draining these different land uses. We sampled nine streams in total: three reference streams draining intact native forest, three draining organic agroforestry farms, and three draining monoculture farms with little or no riparian vegetation. Our research questions were as follows. 1) Are there differences in the benthic communities of streams draining different types of land uses? 2) Which environmental factors explain differences among macroinvertebrate assemblages from streams draining different land uses? 3) Do organic farmlands have fewer impacts on stream biota than palmito monocultures?

4. MATERIALS & METHODS

4.1 *Study area*

The Mashpi river basin is located in the northwestern Andean ridge of Ecuador (Fig 1) eventually becoming part of the Esmeraldas river basin, which drains to the Pacific Ocean. We studied nine streams, all first order tributaries of the Mashpi River at approximately 500 m above sea level. The sites were divided into three categories, based on land use patterns within their drainage areas. The first category included three streams located inside the intact Mashpi forest, composed mostly of pristine montane forests. The second category contained of three streams draining organic agroforestry farms that did not use pesticides and had significant riparian vegetation. The last category contained three streams that drained monoculture palmito (*Bactris gasipaes*) farmland with no natural vegetation cover and little to no riparian vegetation. All streams experienced the same macro-environmental conditions as they were in close proximity (<2km apart) and at similar elevation.

4.2 *Benthic sampling*

The nine streams were sampled twice, once in February 2015 (wet season) and again in May 2015 (dry season). Each stream was divided into three stations along a 50 m length. The sampling stations in each stream were located as follows: one downstream (0m), one in the middle (25m), and one upstream (50m). In each station, we took a qualitative kick sample using a D-net (Hauer and Lamberti 2006). Each kick sample was performed during one minute in which we tried to cover all the microhabitats of the stream. Additionally, in each stream we collected three quantitative samples, one at each station, in riffle habitats using a Surber sampler (500 cm²; mesh size 200 µm) (Surber 1937). Most macroinvertebrates were identified to genus, except for Collembola, Haplotaxida, Isopoda, Trombidiformes,

Unionoida, Basommatophora, Gordioidea and Tricladia, that were identified only to order. The Chironomidae were identified to subfamily. For these identifications, we used specialized keys to North and South American macroinvertebrates (Domínguez and Fernández 2009; Merritt & Cummins 1996; Roldán 1998; Springer *et al.* 2010).

4.3 *Periphyton*

We used chlorophyll *a* concentration as a measure of periphyton biomass, using the spectrophotometric method (Steinman *et al.* 2006; APHA Method 10200H 1998). At each site, three cobbles were collected randomly at each transect. An 4 cm² area of each cobble was scraped and the removed material was filtered with a vacuum pump, using microfiber glass filters, GF/F 47 mm in diameter. We kept the filters at -20°C until one day before extraction; then we put them in 96% ethanol at 4°C for 24 hours to extract the chlorophyll. Then, we centrifuged 15ml of the extract to sediment any impurity in the filter. We measured absorbance at 665 and 750 nm using a spectrophotometer (HACH DK3900, Loveland, CO.). To correct for phaeophytin content, we acidified the extract with 0.5 ml of 0.1N HCL, and measured absorbance again at 665 and 750 nm wavelengths. The quantity of chlorophyll was estimated using the following formula:

$$\text{Chlorophyll } a \text{ (}\mu\text{g/cm}^2\text{)} = 26.7 (E_{664b} - E_{665a}) \times V_{\text{ext}} / A \text{ (cm}^2\text{)} \times L$$

Where 26.7 was the absorption coefficient for chlorophyll *a* in 96% ethanol. E664b was the (absorbance at 664nm - absorbance at 750nm) before the acidification. E664a was the (absorbance at 665nm - absorbance at 750nm) of the acidified sample. V_{ext} was the volume of extract (15ml). A was the area of the stones (4 cm²) and L was the length of the cell used in the spectrophotometer (1 cm).

4.4 *Environmental variables*

At each sampling event, we measured pH, dissolved oxygen (mg/L), conductivity ($\mu\text{S}/\text{cm}$), temperature ($^{\circ}\text{C}$), discharge (m^3/s) and percentage of oxygen, using YSI PRO probes (Yellow Springs USA). For the discharge measurements, we used the salt dilution method (White 1978). This method consists of a bucket with a known amount of dissolved salt (volume and conductivity) added upstream stream then conductivity is measured every 10 seconds at 15 – 25 m downstream. Mean current velocity and discharge was calculated as the time elapsed for half of the salt to pass the stream reach divided by the length of the reach. At each stream, we took 750 ml samples of water that were frozen and taken to the laboratory for measurements of nitrate and phosphate concentrations. We analyzed phosphate concentration using the SRP method (Murphy and Riley 1962), and nitrate concentration using the cadmium reduction method (Henriksen and Selmer-Olsen 1970) with a spectrophotometer (HACH DK3900, Loveland CO.) Table 11.

We characterized the substrate using the pebble count method. The intermediate axis of 100 random sediment particles in each stream habitat was measured (Kondolf and Li 1992). We measured Benthic Coarse Particulate Organic Matter (CPOM) to account for potential differences in food availability for macroinvertebrates. These particles are the main energy source for members of the basal trophic chain in forest streams (Abelho and Graca 1998; Vannote et al. 1980; Cummins et al. 1989). CPOM ($>1\text{mm}$) was collected from Surber samples after all macroinvertebrates were removed. The material was dried at 90°C for 24 hours in an oven, weighed and then combusted in a muffle furnace at 500°C for 4 hours and weighed again to obtain the ash-free dry mass, which was calculated as the difference between the initial and final weight (dried CPOM minus combusted CPOM) (Steinman and Lamberti 1996).

4.5 Data analysis

We assessed the differences and similarities, among and between treatments, using an ANOSIM (Analysis of Similarities) in PrimerV6 (Ivybridge UK). This test is widely used to test spatial differences in community assemblages (Chapman & Underwood 1999). We performed this analysis with data from both the standardized D-net samples (relative abundance, without rare taxa) and Surber samples (density without rare taxa), groups classified as rare where those present in less than 0.5% of the total. The Analysis of Similarity (ANOSIM - one-way) is based on the statistical test R , which varies from -1 to +1, with values closer to 1 representing the largest differences between groups (Clarke and Warwick 2001).

To determine which macroinvertebrates were responsible for differences found between and among stream classification, we performed a SIMPER analysis. This analysis allowed us to pinpoint the macroinvertebrate taxa that characterized each category of stream. In addition, with this analysis we could see which groups were affected or favored by the different types of land use in the stream drainage. We performed SIMPER analyses with data from quantitative (density) and qualitative (relative abundance) samples in PrimerV6.

To visualize in a plot how the community composition differed among streams from the same treatment and streams from different treatments we performed Non-metric Multidimensional Scaling (NMDS) (Clarke and Warwick, 2001) analyses for macroinvertebrate relative abundance and density, excluding rare taxa (present in <10% of samples and <0.2% of total density). The data matrix was transformed using a square root transformation and the similarity matrix was calculated using the Bray Curtis-similarity index (ref). We calculated stress as a measure of the accuracy of the similarity matrix. Stress values

below 0.2 correspond to a reasonable fit (Clarke and Warwick 2001). The NMDS was performed using PrimerV6 (Ivybridge UK).

To account for the effect of environmental variables on community composition, a NMDS analysis of Surber taxa density with environmental fit using all our environmental measured variables. We performed this analysis with the VEGAN package of R (R Development Core Team 2014) (Oksanen *et al.* 2016).

To test if there was any significant difference in the environmental variables among treatments, we used a One-Way ANOVA in SPSS 13.0 for Windows (©SPSS Inc. 2004). , We tested for the homogeneity of variances with a Kolmogorov-Smirnov test performed with the Statistica software (StatSoft Inc. 2007) to satisfy the assumptions of this parametric analysis of variance. This test compared the empirical data against a hypothetical distribution (Gotelli y Ellison 2004). Next, we performed a Principal Component Analysis (PCA) to spatially visualize any differences in the physico-chemical variables of streams within and between treatments. All variables were normalized as a requirement for the use of Euclidean distance as a measure of dissimilarity. We ran the PCA using PrimerV6.

To account for any significant variability in the communities in different categories of streams, we performed a Cross Nested ANOVA in SPSS 13.0 for Windows (©SPSS Inc. 2004). Several community metrics were used: richness (S), abundance (N), density and the Shannon Wiener diversity index (N1) in its exponential form to express the result as true number of species (Jost 2006). These metrics were calculated using Primer v6 (Clarke and Gorley 2006).

5. RESULTS

5.1 *Benthic communities in the Mashpi streams*

We collected 15,615 benthic invertebrates in the nine streams during the two sampling events; 7,468 were captured with the kick D-net method and 8,147 with the Surber sampling method. Specimens were classified into 140 taxa, belonging to 18 different Orders, the majority of which were Coleoptera, with 32 genera, 26 taxa of Diptera, 11 genera of Ephemeroptera and 29 genera of Trichoptera (Table 11). We also present the environmental variables taken during the two months of sampling Table 12.

5.2 *Community differences between land uses*

Streams draining intact forests had the highest similarity in benthic relative abundance (NMDS Cluster 58% similarity; stress = 0.12) (Figure 3); while the streams from organic agroforestry farms and palmito monoculture had a wider, more dispersed, distribution on the plot (Figure 3) indicating higher degrees of heterogeneity. The least similar communities were the ones from streams draining palmito monoculture areas compared with the other two stream categories (palmito compared with forest streams $R = 0.296$, palmito compared with organic agroforestry farms $R = 0.259$, $p = 0.45$) (Table 6). The organic agroforestry farms and forest streams had R values closer to zero ($R = 0.185$; $p = 0.45$) indicating higher similarity in the relative abundance of their invertebrate communities.

The taxa responsible for dissimilarities between streams draining forest and organic agroforestry farms compared with streams draining palmito monoculture farms were mainly *Nectopsyche* sp (Leptoceridae; Trichoptera), *Anacroneuria* sp (Perlidae ;

Plecoptera), *Hyallela* sp. (Hyallelidae; Crustacea) and *Corydalus* sp. (Corydalidae; Megaloptera) (SIMPER Analysis, Table 2). These taxa were not found in streams draining palmito monoculture farms. Additional taxa contributed to differences between streams draining palmito monoculture and the other two stream categories. These included midges (Chironomidae; Orthoclaadiinae) which were more abundant in streams draining palmito monocultures compared to streams draining the other two land-uses. The average abundance of Orthoclaadiinae in palmito monoculture streams was 27 individuals per sample, more than twice those present in forest streams (12) and organic agroforestry farms (7). Other groups with higher relative abundance in palmito monoculture streams compared to streams draining the other two land use categories were *Farrodes* sp. (Leptophlebiidae, Ephemeroptera) and *Pedrowygomyia* sp. (Simuliidae; Diptera). With the Orthoclaadiinae, these taxa comprised almost 50% of the differences between the communities in streams draining palmito monocultures and those draining other land use types. SIMPER analysis showed some taxa declined gradually from organic agroforestry streams to palmito monoculture streams, eg. *Chimarra* sp. (Philopotamidae, Trichoptera) and *Campylocia* sp. (Euthyplociidae; Ephemeroptera). Organic agroforestry streams average abundance: *Chimarra* = 13; *Campylocia* = 5. Monoculture palmito streams average abundance: *Chimarra* = 3; *Campylocia* = 0.

The Non-Metric Dimensional Scaling (NMDS) analysis of benthic invertebrate density (Figure 3) showed an opposite pattern than the NMDS of relative abundance (Figure 2). The cluster analysis of the NMDS grouped all streams draining palmito monoculture areas with a 58% similarity, (stress = 0.12) (Figure 4) The stream draining intact forest areas were not grouped. The remaining streams did not form discrete groups. The ANOSIM of invertebrate densities (Table 7) did not have a significant value to separate the stream

categories $p = 0.2$ similar to the ANOSIM of relative invertebrate abundance, there was a higher dissimilarity of communities in streams draining palmito monocultures compared with the organic agroforestry farms ($r = 0.148$) and the forest streams ($r = 0.259$). The organic agroforestry farms and forest streams had a value close to zero (0.037) suggesting high similarity in the densities of invertebrates in these communities.

The dissimilarity between palmito monoculture streams with the other two categories of streams was lower (41%) than the dissimilarity of streams draining organic agroforestry farms compared with those draining forest streams (43%) (SIMPER Analysis, Table 5). The differences in densities of some taxa were not as marked as the differences in relative abundance between treatments. *Nectopsyche* sp. (Leptoceridae; Trichoptera), *Anacroneuria* sp. (Perlidae; Plecoptera) and *Thraulodes* sp. (Leptophlebiidae; Ephemeroptera) were the major contributors to the density differences among forest streams and streams draining palmito monocultures. The major differences between streams draining organic agroforestry farms and palmito monoculture streams were the absence of *Campylocia* sp. and *Anacroneuria* sp. from palmito monoculture streams.

There was no significant difference in periphyton biomass, estimated through Chlorophyll *a* concentration (Table 7), between treatments ($p = 0.413$). The highest average concentrations in streams draining palmito monoculture lands (mean = 0.13, \pm 0.12 ($\mu\text{g}/\text{cm}^2$)). The forest streams had intermediate values (mean = 0.08, \pm 0.06 ($\mu\text{g}/\text{cm}^2$)) and finally streams draining organic agroforestry farms had the lowest values (mean = 0.04 \pm 0.02 ($\mu\text{g}/\text{cm}^2$)).

There were no significant differences in community diversity metrics (S = richness, N = abundance, $N1$ = Log transformed Shannon Wiener index and density) between the streams

draining different land use types or between the two sampling periods months (see results for the Cross Nested ANOVAs, Table 1; for community metrics see appendix 5).

5.2 Community responses to environmental variables

Water temperatures were significantly higher in the palmito monoculture streams (ANOVA, $p = 0.006$) with an average 21.8°C (Table 10). The average water temperatures of streams draining the organic agroforestry farms and forests were 21.5°C and 21.3°C , respectively. None of the other environmental variables differed significantly between treatments. Please add measures of variation to these mean temperature values.

The Principal Component Analysis (PCA) of the environmental variables showed that Streams draining palmito monocultures had high mean values of phosphates (0.161 ± 0.14 mg/L), nitrates (0.388 ± 0 mg/L) and water temperature $21.8^{\circ}\text{C} \pm 1.24^{\circ}\text{C}$ (Fig 5). Streams draining organic agroforestry farms were more dispersed on the plot, but their characteristic variables were high discharge (0.03 ± 0.02 m³/s) and high levels of CPOM (5.58 ± 1.80 gr). Streams draining intact forests were centered on the plot; they did not have any strong abiotic variable that differentiated them from the other two stream categories (Table 8).

Discharge had a significant influence (NMDS, $p = 0.045$) on the composition of the aquatic communities. The amount of phosphates and nitrates were also strong factors differentiating benthic communities in palmito monoculture streams from benthic communities in the other two categories of streams (Fig 6) (Table 9).

6. DISCUSSION

The goal of this study was to reveal impacts from different land uses on communities of aquatic invertebrates in streams in the Choco biodiversity hotspot. We wanted to see how these communities have changed along a gradient of human impact, from pristine forests to monoculture palmito (*Bactris gasipaes*) plantations. These biodiverse tropical stream communities have apparently changed due to changes in land-use. In general, we found that the invertebrate communities and morphology of streams draining human dominated land-use were different from streams draining lands with lower levels of anthropogenic disturbance. Certain taxa were lost when there was a conversion from forest to agricultural land. The benthic invertebrate communities in stream draining organic agroforestry farms did not differ significantly from intact forest stream communities. Whereas invertebrate communities from palmito monoculture streams were significantly different compared with communities in streams draining intact forests.

6.1 Effects of land use on benthic fauna

One of the most detrimental human practices for rivers and streams is the establishment of agricultural monocultures that eliminate forests to maximize production. This is especially true in small streams, which are among the most threatened habitats due to the extent of land converted to agriculture (Harding *et al.* 1998). Complete forest clearing reduces allochthonous inputs to streams, which results in modifications of their trophic structure (Abelho and Graça 1998). The loss of forest leaf inputs to streams has consequences

for the abundance of shredders but is modified by multiple factors, eg. The type of leaf and the amount of conditioning of the leaves by microorganisms (Golladay *et al.* 1985; Graca 2001). In our study, there was a decline in the relative abundance and density of the shredder *Nectopsyche* sp. in streams draining palmito monocultures. This was probably caused by the lack of diversity in leaf input, together with a reduced fungi community colonizing the leaves as found in other studies (Chergui and Pattee 1991; Graca *et al.* 1993; Kiran 1996, Encalada *et al.* 2010; Scrimgeour and Kendall 2003; Rosenberg and Resh 1993). *Nectopsyche* sp. were important components of the forest streams and declined in streams draining organic agroforestry farms and almost disappeared in streams draining palmito monoculture farmlands. The decline in abundance of Leptocerids (Trichoptera) has been attributed to their sensitivity to aquatic pollutants (Rios-Touma *et al.* 2014), but also to their dependency on different sources of allocthonous material (Wallace *et al.* 1997; Rios-Touma *et al.* 2011). In our study, the shredder functional feeding group (eg. *Phylloicus* sp. and *Nectopsyche* sp., was very important in processing CPOM. These insects accelerate litter fragmentation for other taxa to feed on and produce fecal pellets that contribute to secondary production. The absence of these taxa cascades through the food web and eventually results in less productive streams (Graca 2001; Webster & Benfield 1986; Allan and Castillo 2007).

Other effects of deforestation near streams are an increase in superficial runoff, deposition of fine sediment, pesticides and increased nutrient input, accompanied by higher water temperature (Kasangaki *et al.* 2008; Pringle and Bernstead 2001; Iwata *et al.* 2003a). Several studies have found that plecopterans are very susceptible to organic pollution and lack of dissolved oxygen (Armitage *et al.* 1983; Lenat 1988; Rios-Touma *et al.* 2014). In our study, there was a complete absence of *Anacroneuria* sp. (Plecoptera) from palmito monoculture streams, possibly due to higher water temperature.

Another macroinvertebrate group absent in palmito monoculture streams was the *Campylocia* sp. These detritivorous burrowers ingest large amounts of fine particles deposited in sedimentation areas (Fenoglio *et al.* 2008). This genus of mayflies uses its large mandibles to stay fixed in sediments under stones. This habitat preference may explain its absence in palmito monoculture streams. Stream sediments are usually the main sinks of pollutants that enter streams (Cameron *et al.* 2002, Magbauna *et al.* 2013). Eventhough we did not document the presence of any pesticide in water or sediments, we do know that a lot of herbicide is used and washed off to these streams.

The amphipods *Hyalloela* sp. were also absent from the streams draining palmito monoculture lands but they were found in streams draining organic agroforestry farms. These crustaceans are particularly sensitive to the ingredients present in glyphosate herbicide (Tsui and Chu 2004). We did not examine glyphosate content in running waters in our study because of lack of local suitable laboratories for this analysis. Nevertheless, there is widespread use in the community of this herbicide in roads, near streambeds and of course inside palmito plantations. We had seen empty kegs of herbicide thrown carelessly inside monoculture plantations, some very close to the streams. Local workers of monoculture farmlands informed us of an estimate of 0.2 liters of herbicide dissolved in two liters of water spread per hectare each month, which is inside the limit permitted, by INIAP in Ecuador (2-4 liters per hectare).

Stream communities from palmito monoculture lands were not similar to streams draining the other land uses. This difference was caused mainly by the high numbers of midges, Orthocladiinae. These fly larvae were very abundant in streams draining palmito monoculture lands. The chironomids are a well-known group of insects that are tolerant to environmental extremes with a high tolerance to chemical and organic pollution. They have

high recolonization rates in these habitats, due to their short life cycles and good flight capacity (Armitage *et al.* 2012).

6.2 Variables responsible for community differences

Many studies have shown that human managed intensive agriculture have lower evapotranspiration if compared them with natural vegetation (Canadell *et al.* 1996; Coe *et al.* 2011, Costa *et al.* 2003; Eagleson 1978; Gardner 1983; Li *et al.* 2007; Raymond *et al.* 2008). This is especially true in annual crops and perennial pastures that have reduced root density and depth (Coe *et al.* 2011). The root system in forests also plays a key role in stabilizing stream banks and preventing erosion (e.g., Chamberlin *et al.* 1991, Tabacchi *et al.* 2000). In our study, discharge was highly influential in the distribution patterns of macroinvertebrates. Discharge was higher in streams draining both organic agroforestry farmlands and palmito monoculture streams. This higher discharge probably happens because these streams have a less complex root system in their banks, causing water to enter streams at a higher rate and differentiating the communities of benthic invertebrates in these human impacted streams compared with the forested streams.

The streams draining palmito monocultures were warmer than streams draining forest and agroforestry lands. This could be related to the lack of streamside canopy that exposes streams to high solar radiance, causing higher runoff and making water in these streams warmer and less oxygenated. The Mashpi drainage basin has a high level of solar radiance when there is no cloud cover, making forest cover essential to avoid high temperatures. Other studies have also found that logging in drainage basins leads to an increase in the average water temperatures of streams (Burton and Likens 1973; Holtby and Scrivener 1988, St-Hilaire *et al.* 2000). The absence of Plecoptera in all monoculture streams may be caused by these high temperatures. The immatures require cool water and an abundance of dissolved

oxygen (Jewett 1959). The amount of oxygen that can be dissolved in water is a function of temperature. The lower the temperature, the greater the concentration of O₂ under equilibrium conditions (Dodds 2002).

6.3 Impact differences between monoculture and agroforestry farmlands

Less invasive and destructive agricultural practices (i.e., preservation of buffering vegetation near streams and avoiding water pollution) had lower impacts on diversity and abundance of macroinvertebrates, although no significant difference was found. There were large differences in the abundance of certain groups forming the community composition in streams draining palmito monocultures compared with organic agroforestry farmlands. None of the taxa in the main orders (Plecoptera, Crustacea, Trichoptera and Ephemeroptera) disappeared in streams draining agroforestry lands. In palmito monoculture streams, some taxa were not found (eg., *Anacroneuria* sp., *Hyallela* sp., *Campylocia* sp., *Traverhyphes* sp.). In contrast, in streams draining organic agroforestry farms some taxa increase in abundance eg. *Chimarra* sp., *Campylocia* sp., *Zelus* sp. and *Anacroneuria* sp. in comparison to pristine forest streams, it is possible that these genera are favored by the intermediate disturbance agroforestry organic farms streams cause. *Chimarra* sp. and *Campylocia* sp. are part of the collector-gatherer functional feeding group and, in these streams these genera may be favored by the amount of food material available and less competitors than in mature and established communities of pristine forest streams (Wiggins 1996, Wiggins 2004; Tomanova *et al.* 2006; Reynaga 2009). *Anacroneuria* are facultative predators (Merritt & Cummins 1996; Tomanova *et al.* 2006; Reynaga and Rueda 2010). In biologically diverse streams with diverse substrates such as the forest streams, they are able to exploit a variety of prey types.

7. CONCLUSIONS

Deforestation near streams affects the presence of some Trichoptera, all Plecoptera and all Amphipoda, and potentiates the colonization of tolerant Diptera, Chironomidae. Streams of the area were relatively well conserved, and they have high diversity. The main threats to this diversity are deforestation, sedimentation, and presumably, chemical and organic pollution. These streams are the source of water for many people in lowlands and should be a focus for conservation efforts and research. We found that even small changes in land use could lead to local extinction of some groups of benthic macroinvertebrates.

The lack of significance in the community metrics (Diversity (S), Abundance (N), Shannon (N1) & Density) in stream draining different land uses types may be caused by: 1) diversity of these rivers is high enough that the differences can only be seen in certain taxa; 2) these communities may be resilient to negative impacts because of high diversity and high density of the macrobenthos in streams permit constant recolonization.

To have a better understanding of the effect of deforestation and pesticides in these streams, it would be necessary to perform mesocosmos experiments controlling emergence success and drift with controlled exposures to herbicides and organic material availability

that mimic conditions seen in the streams. *Hyalpella* sp., *Anacroneuria* sp., *Campylocia* sp., *Corydallus* sp. and other taxa absent from the palmito monoculture streams would be interesting to study in these controlled experiments to differentiate the effects of deforestation from pesticide exposure. The effect of the possible presence of pesticides, especially herbicides, were not obvious in the community diversity and abundance metrics. The effects of chronic exposure can only be seen clearly by studying the life history of macroinvertebrates including emergence success. This is especially true when other studies link glyphosate exposure with an increase in drift and emergence propensities of just a few glyphosate-sensitive taxa. This herbicide can also reduce the size and success rate of emerging adult insects (Magbanua *et al.* 2013).

8. REFERENCES

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9. FIGURES

Figure 1. Topographic map of the Mashpi river subbasin in Ecuador, showing the 9 studied streams, monoculture plantations of the zone and the protected rain forest area.

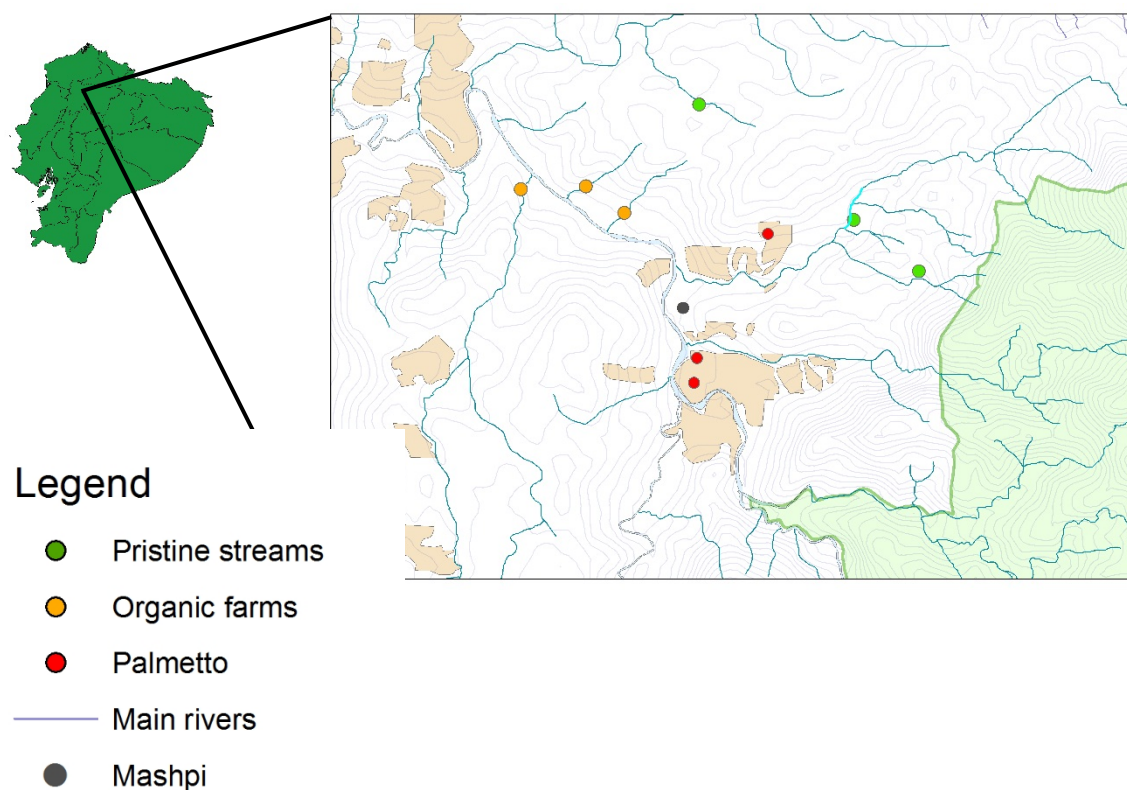


Fig 2. Non- Metric Multidimensional Scaling using average cluster similarity for stream relative abundance of macroinvertebrate communities draining different land uses in Mashpi basin, Ecuador. Data was previously transformed using a fourth root transformation and resemblance table was performed using the Bray Curtis Similarity index.

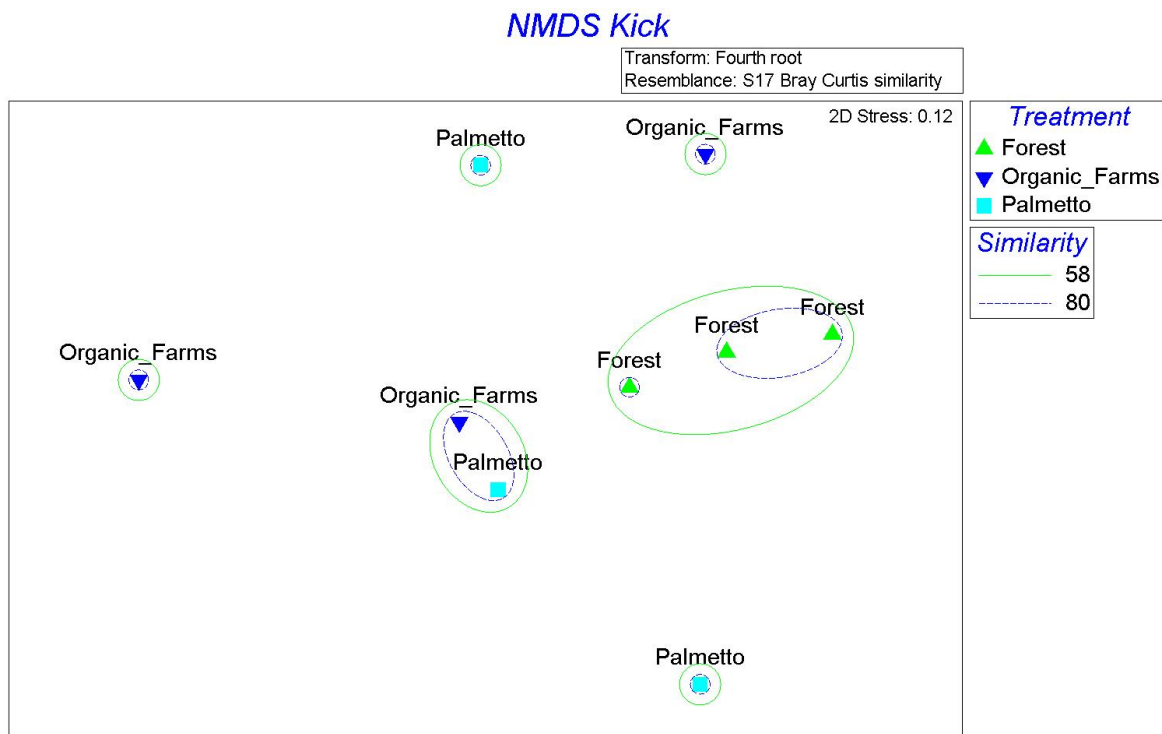


Fig 3. Non- Metric Multidimensional Scaling using average cluster similarity for stream density of macroinvertebrate communities draining different land uses in Mashpi basin, Ecuador. Data was previously transformed using a fourth root transformation and resemblance table was performed using the Bray Curtis Similarity index.

NMDS Surber

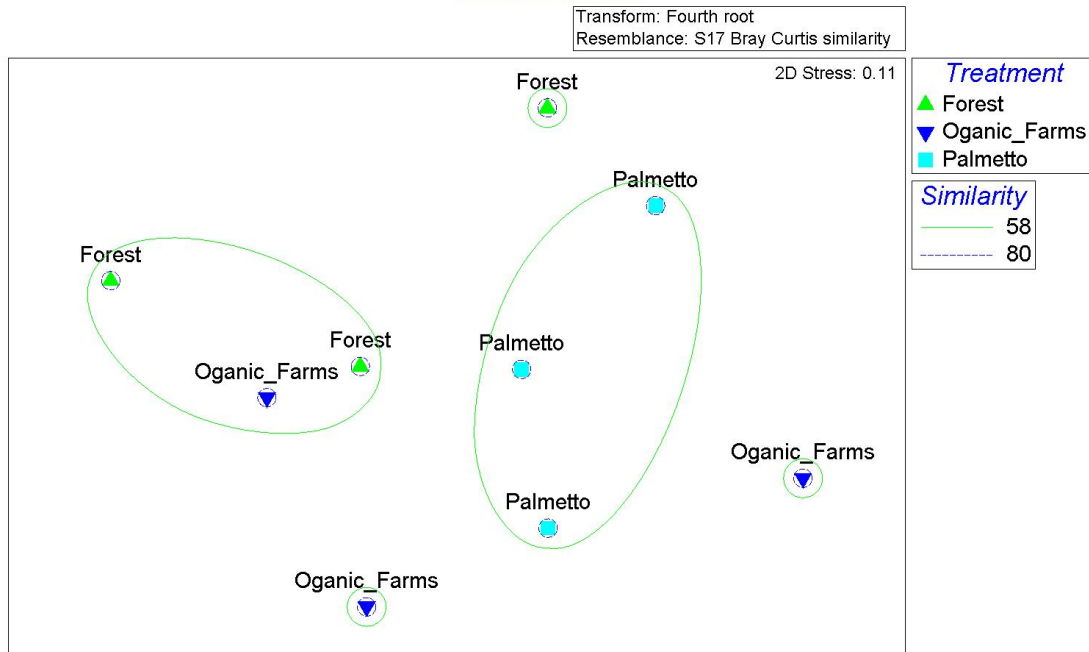


Fig 4. PCA with environmental data differences of the nine streams studied in the Mashpi basin, Ecuador. Streams were grouped by treatment and distanced with a 3.1 Euclidean distance. Data was previously transformed using a fourth root transformation and clustered.

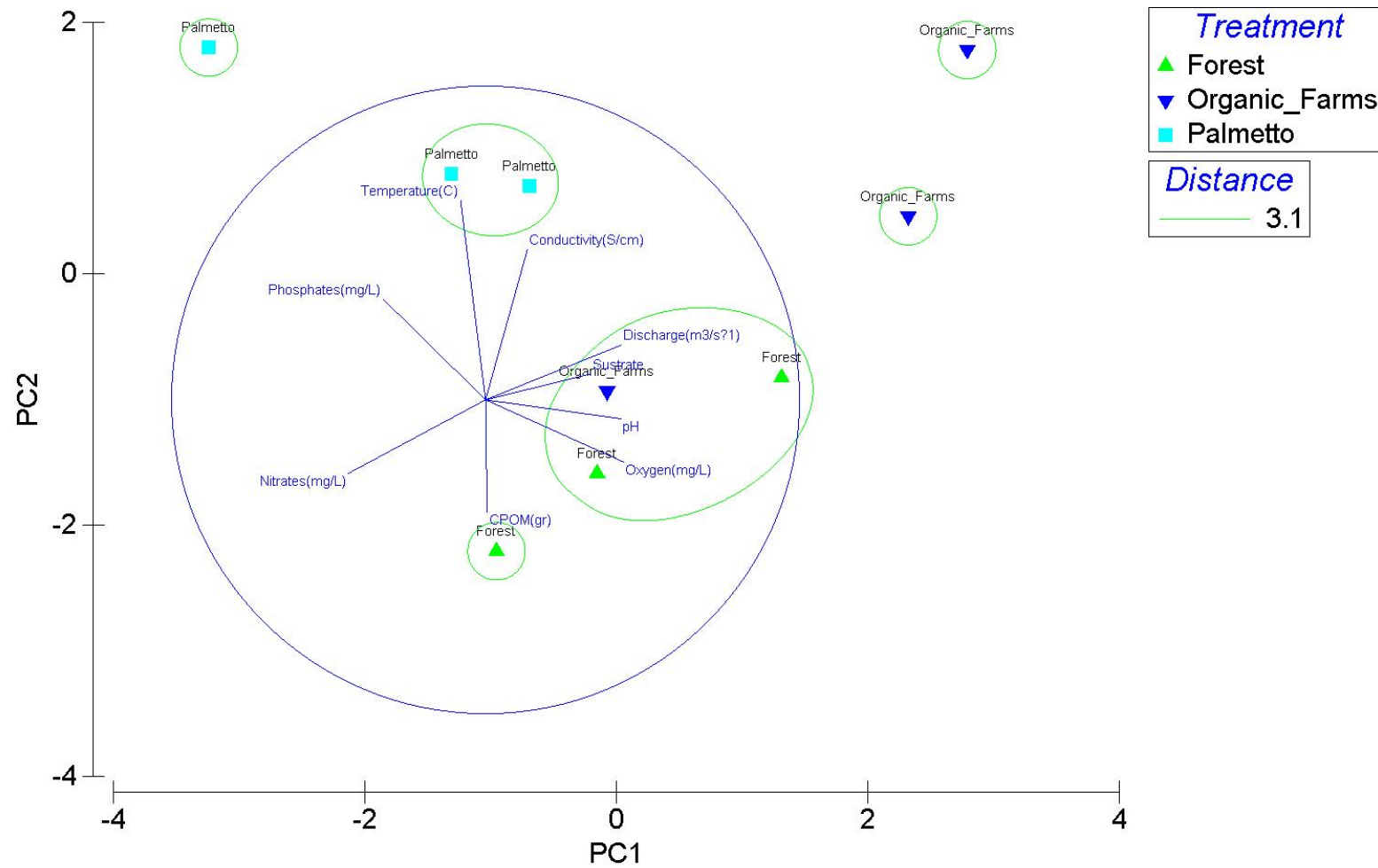
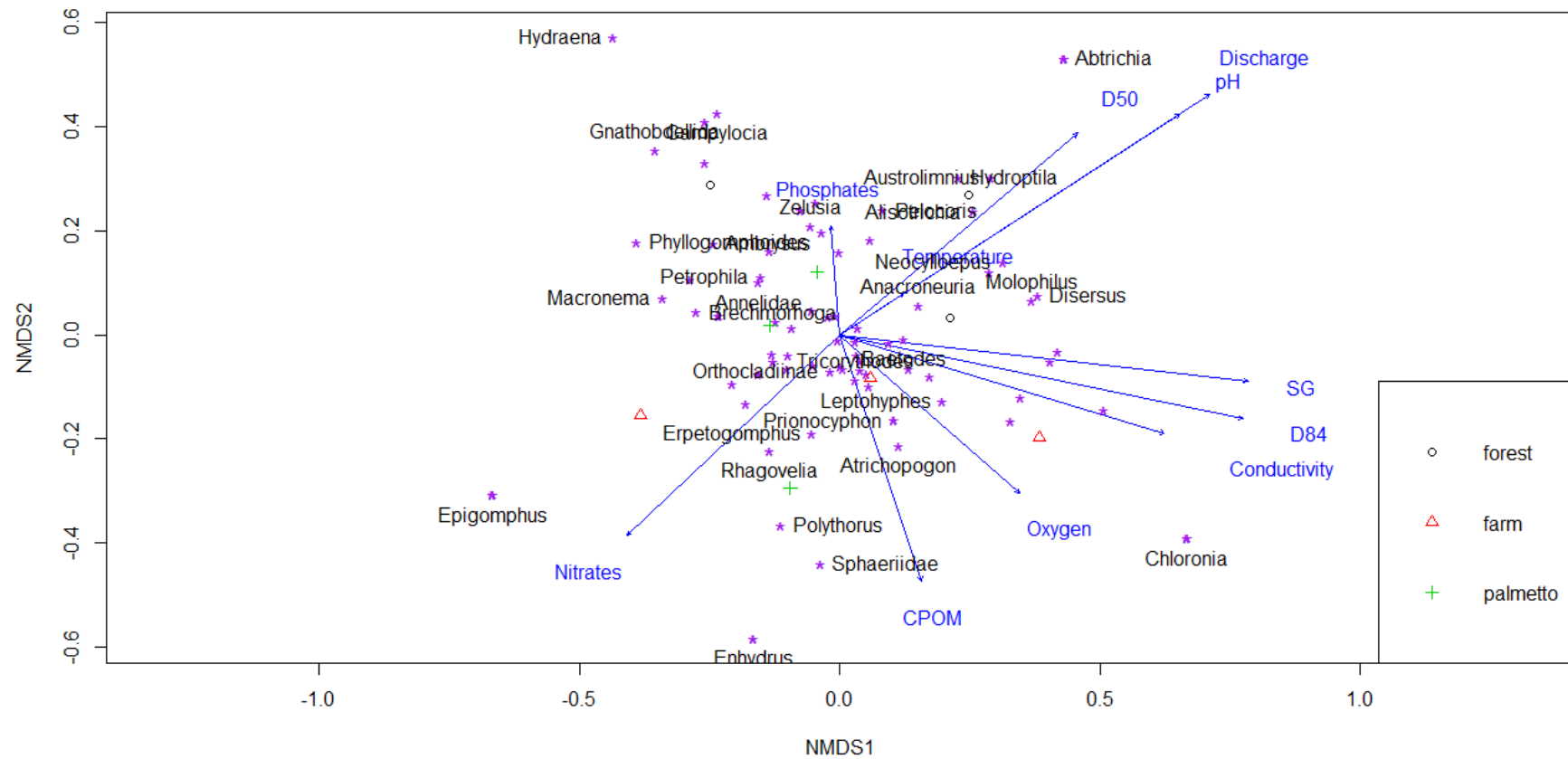


Fig 5. NMDS with environmental Fit performed on R package with Vegan software. Environmental variables weight in taxa distribution of the nine streams studied in the Mashpi basin, Ecuador. Streams were grouped by treatment type. Each star represent a different taxa.



10. TABLES

Table 1. Cross nested ANOVAs results of community metrics of all nine stream macroinvertebrate communities in the Mashpi river watershed, Ecuador. R values of differences among treatments, months and both.

Variable	p values of Cross Nested ANOVAs		
	Treatment	Month	Month & Treatment
Richness (S)	0.94	0.579	0.324
Relative Abundance (N)	0.38	0.093	0.182
Density	0.38	0.093	0.182
Shannon Wiener (N1)	0.344	0.385	0.55
Total Abundance	0.759	0.113	0.239

Table 2. Similarity percentages (SIMPER) of relative abundance among streams of the same land use. Similarity was calculated using the Bray Curtis similarity index. Presented results are the ones that had at least a 50% cumulative contribution to the similarity among same treatment communities.

2.1 Similarity percentages among Pristine Forest streams.

Group Forest

Average similarity: 49.44

Species	Av.Abund	Av.Sim	Sim/SD	Contrib%	Cum.%
Smicridea	14.11	6.38	2.66	12.91	12.91
Pedrowygomia	14.61	6.14	2.87	12.42	25.33
Anchytarsus	12.61	5.37	1.46	10.86	36.18
Farrodes	13.92	4.28	1.54	8.66	44.84
Orthocladinae	12.22	3.89	2.27	7.87	52.71

2.2 Similarity percentages among Agroforestry Organic Farm streams.

Group Farm

Average similarity: 46.91

Species	Av.Abund	Av.Sim	Sim/SD	Contrib%	Cum. %
Pedrowygomyia	13.17	8.61	3.57	18.36	18.36
Farrodes	20.03	4.86	1.04	10.37	28.72
Orthocladinae	7.39	3.68	1.98	7.85	36.57
Heterelmis	5.86	3.19	2.13	6.8	43.37
Smicridea	6.89	3.02	1.9	6.44	49.81
Chironominae	6.36	2.42	1.58	5.16	54.97

2.3 Similarity percentages among N

Group Pesticides

Average similarity: 39.85

Species	Av.Abund	Av.Sim	Sim/SD	Contrib%	Cum. %
Orthocladinae	26.83	6.48	1.56	16.26	16.26
Farrodes	29.83	5.33	0.96	13.38	29.65
Pedrowygomyia	16.25	5.31	1.29	13.34	42.98
Smicridea	7.67	3.65	3.16	9.17	52.15

Table 3. Dissimilarity percentages (SIMPER) of relative abundance between streams of different land use. Dissimilarity was calculated using the Bray Curtis similarity index.

Presented results are the ones that had at least a 50% cumulative contribution to the dissimilarity between treatment communities.

3.1 Compared dissimilarities between Pristine Forest streams and Agroforestry Organic Farm streams.

<i>Groups Forest &</i>
<i>Agroforestry Organic Farm</i>
Average dissimilarity =
54.35

	Group Forest	Group Farm				
Species	Av.Abund	Av.Abund	Av.Diss	Diss/SD	Contrib%	Cum.%
Farrodes	13.92	20.03	5.61	1.23	10.33	10.33
Nectopsyche	15.33	1.11	5.09	0.93	9.37	19.7
Chimarra	1.78	13.28	3.5	0.75	6.44	26.14
Anchytarsus	12.61	8	3.28	1.46	6.04	32.17
Smicridea	14.11	6.89	2.94	1.28	5.42	37.59
Orthocladinae	12.22	7.39	2.71	1.12	4.98	42.57
Pedrowygomyia	14.61	13.17	2.27	0.99	4.17	46.74
Chironominae	7.44	6.36	2.18	0.93	4.01	50.75

3.2 Compared dissimilarities between Pristine Forest streams and Monoculture streams.

<i>Groups Forest & Pesticides</i>
Average dissimilarity = 57.07

Species	Group Forest	Group Pesticides	Av.Diss	Diss/SD	Contrib%	Cum. %
	Av.Abund	Av.Abund				
Farrodes	13.92	29.83	7.09	1.19	12.42	12.42
Orthocladinae	12.22	26.83	5.95	1.19	10.43	22.84
Nectopsyche	15.33	0.83	4.77	0.9	8.36	31.2
Pedrowygomyia	14.61	16.25	3.68	1.32	6.45	37.65
Chironominae	7.44	13.22	3.28	0.92	5.75	43.41
Anchytarsus	12.61	10.69	2.89	1.62	5.06	48.47
Smicridea	14.11	7.67	2.43	1.13	4.26	52.73

3.3 Compared dissimilarities between Agroforestry Organic Farms streams and Monoculture streams.

<i>Groups Farm & Pesticides</i>
Average dissimilarity = 57.02

Species	Group Farm	Group Pesticides	Av.Diss	Diss/SD	Contrib%	Cum. %
	Av.Abund	Av.Abund				
Farrodes	20.03	29.83	8.37	1.32	14.67	14.67
Orthocladinae	7.39	26.83	5.94	1.11	10.41	25.09
Chimarra	13.28	3.17	3.69	0.84	6.48	31.56
Chironominae	6.36	13.22	3.31	0.92	5.81	37.37
Pedrowygomyia	13.17	16.25	3.14	1.36	5.5	42.87
Anchytarsus	8	10.69	2.68	1.45	4.69	47.56
Campylocia	5.28	0	1.57	1.02	2.76	50.32

Table 4. Similarity percentages (SIMPER) of densities among streams of the same land use.

Similarity was calculated using the Bray Curtis similarity index. Presented results are the ones that had at least a 50% cumulative contribution to the similarity among same treatment communities.

4.1 Similarity percentages among Pristine Forest streams.

<i>Group Forest</i> Average similarity: 58.48					
Species	Av.Abund	Av.Sim	Sim/SD	Contrib%	Cum.%
Smicridea	2.86	4.31	35.58	7.37	7.37
Heterelmis	2.11	3.14	25.41	5.37	12.74
Alluaudomyia	2.13	3.03	6.15	5.18	17.92
Palaemnema	2.02	2.92	14.7	4.99	22.9
Leptonema	2.07	2.82	8.88	4.82	27.72
Tanypodinae	1.87	2.54	4.95	4.34	32.06
Baetodes	1.9	2.51	6.62	4.29	36.35
Limnophila	2.02	2.45	3.89	4.19	40.55
Tricorythodes	1.48	2.09	5.94	3.57	44.11
Chimarra	1.74	2.02	2.51	3.45	47.56
Atopsyche	1.41	2	14.7	3.42	50.99

4.2 Similarity percentages among Agroforestry Organic Farm streams.

<i>Group Organic_Farms</i> Average similarity: 57.30					
Species	Av.Abund	Av.Sim	Sim/SD	Contrib%	Cum.%
Smicridea	2.42	3.93	7.5	6.85	6.85
Alluaudomyia	1.83	3.06	13.2	5.33	12.18
Heterelmis	1.95	2.87	4.61	5.01	17.19
Chimarra	2.15	2.81	5.49	4.9	22.09
Tanypodinae	1.83	2.81	5.49	4.9	26.99
Baetodes	2.08	2.58	2.74	4.5	31.49
Limnophila	1.93	2.57	4.51	4.49	35.98
Anacroneuria	1.64	2.43	4.4	4.24	40.22

Planariidae	1.52	2.37	13.2	4.14	44.37
Zelus	1.76	2.31	4.72	4.03	48.4
Mortoniella	1.34	2.12	11.24	3.69	52.09

4.3 Similarity percentages among Monoculture streams.

Group
Palmetto
Average
similarity:
60.31

Species	Av.Abund	Av.Sim	Sim/SD	Contrib%	Cum.%
Tanypodinae	2.67	4.46	7.7	7.39	7.39
Smicridea	2.62	4.4	9.31	7.29	14.67
Limnophila	2.54	4.02	4.75	6.67	21.34
Chimarra	2.24	3.53	17.5	5.85	27.19
Tricorythodes	2.12	3.43	11.35	5.69	32.88
Leptonema	2.01	3.28	9.28	5.43	38.32
Hexatoma	1.89	3.05	8.26	5.05	43.37
Alluaudomyia	2.08	3.03	7.02	5.02	48.39
Planariidae	2.14	2.9	12.12	4.8	53.2

Table 5. Dissimilarity percentages (SIMPER) of densities between streams of different land use. Dissimilarity was calculated using the Bray Curtis similarity index. Presented results are the ones that had at least a 50% cumulative contribution to the dissimilarity between treatment communities.

5.1 Compared dissimilarities between Pristine Forest streams and Agroforestry Organic Farm streams.

Groups Forest & Organic_Farms						
Average dissimilarity = 43.29						
Species	Group Forest	Group Organic_Farms	Group			
			Av.Abund	Av.Diss	Diss/SD	Contrib%
Species	Av.Abund	Av.Abund	Av.Diss	Diss/SD	Contrib%	Cum.%
Campylocia	0	1.94	1.6	1.79	3.7	3.7
Nectopsyche	2.2	0.89	1.55	1.67	3.57	7.27
Polythorus	1.33	0	1.08	5.16	2.5	9.77
Zelus	0.4	1.76	1.08	1.89	2.49	12.26
Palaemnema	2.02	0.73	1.06	2.05	2.45	14.71
Pelocoris	0.33	1.36	0.83	1.97	1.92	16.63
Erpetogomphus	1	0	0.82	14.26	1.89	18.52
Neelmis	1.37	0.4	0.82	1.56	1.88	20.4
Austrolimnius	0	1.02	0.81	1.32	1.86	22.27
Tricorythodes	1.48	1.41	0.77	1.46	1.77	24.04
Thraulodes	1.08	0.5	0.75	1.12	1.74	25.77
Macronema	0.59	0.89	0.74	1.33	1.7	27.47
Cylloepus	0.94	1.19	0.72	1.15	1.66	29.13
Neocylloepus	0.47	1	0.69	1.1	1.58	30.71
Notelmis	0.67	0.59	0.67	1.41	1.54	32.26
Tubificidae	0.77	1.06	0.67	1.31	1.54	33.79
Lymnaeidae	0.4	0.89	0.66	1.17	1.52	35.32
Hyalala	0.62	0.56	0.66	0.88	1.52	36.84
Tholymis	0.67	1.02	0.65	1.62	1.5	38.34
Phylloicus	1.13	0.33	0.64	1.57	1.47	39.8
Chimarra	1.74	2.15	0.63	1.32	1.46	41.27
Corydalus	0.33	0.84	0.61	1.21	1.41	42.68
Hexatoma	1.14	1.31	0.61	1.42	1.4	44.08
Alisotrichia	0.33	0.83	0.58	1.18	1.34	45.41

Disersus	0.4	0.84	0.57	1.1	1.31	46.73
Macrelmis	0.52	0.5	0.57	0.86	1.31	48.04
Helicopsyche	0.77	0.4	0.56	1.15	1.29	49.32
Leptohyphes	0.33	0.56	0.56	0.91	1.29	50.61

5.2 Compared dissimilarities between Pristine Forest streams and Monoculture streams.

<i>Groups Forest & Palmetto</i>						
Average dissimilarity = 41.94						
Species	Group Forest Av.Abund	Group Palmetto Av.Abund	Av.Diss	Diss/SD	Contrib%	Cum.%
Nectopsyche	2.2	1.24	1.49	1.4	3.55	3.55
Anacroneuria	1.6	0	1.31	3.4	3.11	6.66
Palaemnema	2.02	0.8	0.97	2.08	2.3	8.96
Argia	1.31	0.59	0.87	1.96	2.08	11.05
Thraulodes	1.08	0	0.87	1.32	2.08	13.13
Zelus	0.4	1.41	0.86	1.67	2.06	15.19
Tholymis	0.67	1.18	0.77	1.67	1.83	17.02
Polythorus	1.33	0.44	0.75	1.68	1.8	18.82
Brechmorhoga	0.44	1.02	0.7	1.15	1.66	20.48
Etheriidae	0.33	0.94	0.7	1.21	1.66	22.14
Ambrysus	0	0.8	0.69	1.31	1.64	23.78
Neoelmis	1.37	1.02	0.69	1.54	1.64	25.42
Hexatoma	1.14	1.89	0.68	1	1.63	27.05
Pelocoris	0.33	0.97	0.68	1.36	1.63	28.68
Atrichopogon	1.2	0.4	0.66	1.59	1.58	30.25
Tanypodinae	1.87	2.67	0.66	2.15	1.57	31.83
Lymnaeidae	0.4	0.96	0.66	1.21	1.57	33.4
Erpetogomphus	1	0.44	0.65	2.05	1.56	34.96
Planariidae	1.44	2.14	0.61	1.11	1.46	36.42
Mortoniella	0.77	0.99	0.61	1.2	1.45	37.87
Rhagovelia	0.33	0.86	0.6	1.19	1.44	39.3
Hemerodromia	0.73	0	0.59	1.3	1.4	40.71
Psephenus	0.67	0.5	0.58	1.4	1.39	42.1
Petrophila	0.44	0.84	0.58	1.08	1.37	43.47
Cylloepus	0.94	0.87	0.57	0.98	1.35	44.82
Chelifera	0.33	0.8	0.56	1.18	1.34	46.15
Limnophila	2.02	2.54	0.56	1.24	1.33	47.48
Plectomacronema	0.68	0	0.56	0.66	1.33	48.81
Prionocyphon	0.7	0	0.55	0.66	1.31	50.11

5.3 Compared dissimilarities between Agroforestry Organic Farms streams and Monoculture streams.

Groups Organic_Farms & Palmetto

Average dissimilarity = 41.71

Species	Group Organic_Farm s	Group Palmetto	Av.Diss	Diss/SD	Contrib%	Cum. %
	Av.Abund	Av.Abund				
Anacroneuria	1.64	0	1.41	4.23	3.37	3.37
Campylocia	1.94	0.33	1.34	1.37	3.22	6.59
Tricorythodes	1.41	2.12	0.93	1.31	2.22	8.81
Nectopsyche	0.89	1.24	0.85	1.33	2.04	10.85
Neocylloepus	1	0	0.83	1.31	1.98	12.84
Brechmorhoga	0	1.02	0.82	1.27	1.96	14.8
Etheriidae	0	0.94	0.81	1.21	1.95	16.75
Macronema	0.89	0	0.79	1.29	1.89	18.64
Cylloepus	1.19	0.87	0.77	1.21	1.84	20.48
Neelmis	0.4	1.02	0.75	1.2	1.8	22.29
Tholymis	1.02	1.18	0.75	1.15	1.79	24.08
Austrolimnius	1.02	0.4	0.73	1.22	1.76	25.84
Rhagovelia	0	0.86	0.71	1.3	1.71	27.55
Tanypodinae	1.83	2.67	0.7	3.98	1.69	29.24
Gordoidea	1.13	0.4	0.68	1.41	1.64	30.88
Argia	0.67	0.59	0.67	1.49	1.61	32.49
Lymnaeidae	0.89	0.96	0.66	1.13	1.59	34.07
Ambrysus	0.5	0.8	0.66	1.26	1.57	35.65
Phylloicus	0.33	0.92	0.65	1.31	1.57	37.22
Corydalus	0.84	0.4	0.62	1.07	1.49	38.71
Chelifera	0.44	0.8	0.62	1.2	1.48	40.19
Helicopsyche	0.4	0.83	0.62	1.17	1.47	41.66
Disersus	0.84	0.33	0.61	1.2	1.47	43.13
Petrophila	0.44	0.84	0.6	1.1	1.43	44.56
Alisotrichia	0.83	0.33	0.6	1.17	1.43	45.99
Hydrachnidae	0.98	1.45	0.59	1.13	1.42	47.42
Leptohyphes	0.56	0.33	0.59	0.97	1.41	48.83
Limnophila	1.93	2.54	0.59	1.28	1.4	50.24

Table 6. ANOSIM (Analisis of Similarities) performed with relative abundance kick samples. $R = 0.012$, $p = 0.45$

Groups	R Statistic	Significance Level %	Possible Permutations	Actual Permutations	Number >= Observed
Forest, Organic_Farms	0.185	20	10	10	2
Forest, Palmetto	0.296	20	10	10	2
Organic_Farms, Palmetto	-0.259	90	10	10	9

Table 7. ANOSIM (Analisis of Similarities) performed with densities surber samples. $R = 0.144$; $p = 0.2$

Groups	R Statistic	Significance Level %	Possible Permutations	Actual Permutations	Number >= Observed
Forest, Organic_Farms	0.037	50	10	10	5
Forest, Palmetto	0.259	20	10	10	2
Organic_Farms, Palmetto	0.148	40	10	10	4

Table 8. Peryphiton quantification of each month (AOF = Agroforestry Organic Farms). Streams are grouped by type of land use.

River	Treatment	Month Clorophyll a ($\mu\text{g}/\text{cm}^2$)	
		February	May
Boshungo	Forest	0.02	0.05
Chakra	Forest	0.24	0.07
Maltrib	Forest	0.07	0.05
Ines	AOF	0.04	0.02
Mashung	AOF	0.03	0.06
Pamb	AOF	0.03	0.07
Taipest	Monoculture	0.50	0.03
Mastrib1	Monoculture	0.04	0.01
Mastrib2	Monoculture	0.12	0.11

Table 9. Eigenvalues and eigenvectors explaining main axis of PCA from abiotic variables made on Primer v6.

Eigenvalues					
PC	Eigenvalues	%Variation	Cum.%Variation		
1	3.57	39.6	39.6		
2	2.08	23.2	62.8		
3	1.62	18	80.8		
4	0.926	10.3	91.1		
5	0.494	5.5	96.6		

Eigenvectors					
(Coefficients in the linear combinations of variables making up PC's)					
Variable	PC1	PC2	PC3	PC4	PC5
Temperature(C)	-0.08	0.637	-0.081	0.349	0.052
Conductivity(S/cm)	0.133	0.478	-0.453	-0.031	0.5
Oxygen(mg/L)	0.44	-0.198	0.161	0.099	0.514
pH	0.43	-0.06	-0.25	-0.361	-0.156
Sustrate	0.336	0.083	-0.386	0.345	-0.631
Discharge(m3/s?1)	0.433	0.175	0.137	-0.466	-0.105
CPOM(gr)	0.005	-0.358	-0.644	-0.082	0.19
	-				
Nitrates(mg/L)	0.437	-0.234	-0.338	-0.061	0.01
	-				
Phosphates(mg/L)	0.326	0.32	-0.051	-0.624	-0.121

Table 10. Eigenvalues of NMDS with environmental fit made on R, vegan package.

	NMDS1	NMDS2	r2	Pr (>r)
Temperature	0.837	0.545	0.018	0.939
Conductivity	0.956	-0.291	0.367	0.236
Oxygen	0.749	-0.661	0.183	0.547
pH	0.838	0.545	0.525	0.118
D50	0.7627	0.646	0.31	0.353
D84	0.979	-0.203	0.5416	0.095
SG	0.993	-0.112	0.539	0.103
Discharge	0.838	0.545	0.6233	0.045
CPOM	0.314	-0.94931	0.214	0.516
Nitrates	-0.727	-0.686	0.274	0.377
Phosphates	-0.079	0.996	0.384	0.878

signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Table 11. One way ANOVA p values of all environmental variable differences between treatment. Streams were grouped under type of land use.

Variable	P values
Temperature	0.006
Conductivity	0.748
Oxygen	0.138
pH	0.027
Discharge	0.065
CPOM	0.133
Nitrates	0.073
Phosphates	0.476
D50	0.631
D84	0.885
Sg	0.813

Table 12. All microinvertebrates identified classified under Order. Raw data was separated between months of sampling.

Site	Amphipoda	Bassomato	Coleoptera	Collembola	Diptera	Diptera	Ephemeroptera	Gordioidea	Haplotoxida	Hemiptera	Isopoda	Lepidoptera	Megaloptera	Odonata	Plecoptera	Trichoptera	Tricladida	Trombidiformes	Unionoida	Total
Boshungo	0	0	161	9	545	0	161	1	5	6	0	5	3	59	1	198	4	6	0	1165
February	0	0	126	6	406	0	109	0	4	5	0	5	0	45	1	111	4	3	0	825
May	0	0	35	3	139	0	52	1	1	1	0	0	3	14	0	87	0	3	0	340
Chakra	0	7	450	0	668	0	246	0	1	4	2	0	5	76	21	585	17	7	1	2090
February	0	5	166	0	129	0	51	0	1	1	1	0	2	24	7	303	3	4	0	697
May	0	2	284	0	539	0	195	0	0	3	1	0	3	52	14	282	14	3	1	1393
Ines	0	14	149	1	460	0	290	2	5	20	0	4	4	22	6	102	5	9	0	1094
February	0	14	89	0	249	0	182	0	5	11	0	4	2	9	1	50	5	6	0	628
May	0	0	60	1	211	0	108	2	0	9	0	0	2	13	5	52	0	3	0	466
Maltrib	43	0	362	3	765	0	176	1	6	7	0	0	1	54	22	500	15	14	0	1969
February	32	0	148	1	309	0	97	0	5	3	0	0	0	24	12	263	1	5	0	900
May	11	0	214	2	456	0	79	1	1	4	0	0	1	30	10	237	14	9	0	1069
Mashungo	33	14	280	2	425	0	720	2	16	27	0	0	5	26	34	438	26	4	0	2054
February	14	8	123	1	196	0	294	0	10	9	0	0	0	14	8	136	11	3	0	827
May	19	6	157	1	229	0	426	2	6	18	0	0	5	12	26	302	15	1	0	1227
Maspest1	0	6	354	3	1167	0	670	2	9	27	0	8	2	65	0	225	15	12	17	2584
February	0	6	270	0	338	0	59	0	7	14	0	7	2	25	0	94	8	9	17	857
May	0	0	84	3	829	0	611	2	2	13	0	1	0	40	0	131	7	3	0	1727
Maspest2	0	0	157	5	940	0	346	0	3	11	0	0	0	34	0	152	22	4	7	1681
February	0	0	21	1	230	0	61	0	1	6	0	0	0	7	0	40	4	2	7	380
May	0	0	136	4	710	0	285	0	2	5	0	0	0	27	0	112	18	2	0	1301
Pamb	0	0	214	3	340	136	222	1	1	6	0	0	5	7	17	192	4	4	0	1153
February	0	0	108	1	276	0	108	0	1	4	0	0	2	6	10	117	4	2	0	640
May	0	0	106	2	64	136	114	1	0	2	0	0	3	1	7	75	0	2	0	513
Taapest	0	17	260	3	573	0	400	0	4	18	0	10	0	59	0	273	99	13	0	1731
February	0	9	70	0	334	0	139	0	1	6	0	4	0	34	0	77	8	0	0	684
May	0	8	190	3	239	0	261	0	3	12	0	6	0	25	0	196	91	13	0	1047
Total	76	58	2387	29	5883	136	3231	9	50	126	2	27	25	402	101	2665	207	73	25	15521

Table 13. Environmental data collected separated in treatments and date of measurement. Data collected of Temperature, Conductivity, Dissolved Oxygen, pH, Discharge, Stone sediment diameters (D16, D50, D84 and sg), CPOM, Nitrates and Phosphates.

Treatment	Date	Site	Temperature(°C)	Conductividad Especifica	Conductividad	Oxígeno (mg/L)	pH	Caudal	D16	D50	D84	sg	Discharge	CPOM	Nitratos	Fosfatos
Farms	May	Ines	24.5	34.6	34.3	5.69	7.74	0.069	7	23	63	3.02105442	0.069	4.8	0.3	0.06
Farms	May	Pambiliño	23.2	57.6	64.6	6.41	7.86	0.035	7	19	51	2.70141545	0.035	5.6	0.3	0.23
Farms	May	Mashpi Shungo	23.2	31	30	5.2	7.77	0.010	7	20	46	2.56979105	0.010	7.8	0.4	0.13
Farms	February	Inés	21.5	33.7	32.5	7.26	7.86	0.045	7	23	63	3.02105442	0.045	3.0	0.3	0.06
Farms	February	Pambiliño	20.2	68.1	64	7.6	7.77	0.038	7	19	51	2.70141545	0.038	7.4	0.3	0.23
Farms	February	Mashpi Shungo	21.4	32.9	31.6	7.01	7.38	0.008	7	20	46	2.56979105	0.008	4.9	0.4	0.13
Forest	May	Chakra	22.6	45.8	43.9	6.45	7.78	0.019	7	16	79	3.37225943	0.019	15.2	0.4	0.26
Forest	May	Malimpia Tributario	22.2	47.2	44.7	16.6	7.74	0.015	7	23	47	2.60227564	0.015	9.3	0.5	0.19
Forest	May	Bosque Shungo	22.4	21.1	20	5.29	7.38	0.019	7	13	32	2.12587745	0.019	3.46	0.4	0.11
Forest	February	Chakra	20.1	51.2	48.2	7.5	7.78	0.025	7	20	42	2.46304259	0.025	4.5	0.4	0.26
Forest	February	Malimpia Tributario 1	20.5	48	45.4	6.93	7.74	0.007	7	28	67	3.10127229	0.014	24.1	0.5	0.19
Forest	February	Bosque Shungo	20	21.5	20.4	7.4	7.39	0.003	7	13	32	2.12587745	0.003	10.0	0.4	0.11
Monoculture	May	Taipest	22.9	45.7	44	6.14	7.39	0.007	7	9	28	2.00214672	0.007	5.7	0.4	0.32
Monoculture	May	Mashpi Tributario 1	22.8	45	43.2	7.06	7.39	0.005	7	28	67	3.10127229	0.005	5.1	0.4	0.07
Monoculture	May	Mashpi Tributario 2	22.9	49.1	47.2	6.25	7.39	0.003	7	20	42	2.46304259	0.003	10.0	0.4	0.12
Monoculture	February	Mashpi Tributario 1	20.7	48	46	6.85	7.39	0.004	7	9	28	2.00214672	0.004	5.9	0.4	0.32
Monoculture	February	Mashpi Tributario 2	20.6	46.6	44.2	7.04	7.39	0.001	7	16	79	3.37225943	0.001	4.4	0.4	0.07
Monoculture	February	Taipest 1	20.5	52.1	49.5	6.34	7.39	0.007	7	23	47	2.60227564	0.007	5.4	0.4	0.12

11. Appendices

Appendix 1. Cross nested Anova results of community metrics performed on SPSS with significance values.

Tests of Between-Subjects Effects

Dependent Variable: Total Individuals (N)

Source		Type III Sum of Squares	df	Mean Square	F	Sig.
Treatment	Hypothesis	42,535,815	2	21,267,907	1,141	,380
	Error	111,838,222	6	18639,704 ^a		
Treatment * Site	Hypothesis	111,838,222	6	18,639,704	2,929	,108
	Error	38,185,111	6	6364,185 ^b		
Month	Hypothesis	25,306,685	1	25,306,685	3,976	,093
	Error	38,185,111	6	6364,185 ^b		
Treatment * Month	Hypothesis	29,226,037	2	14,613,019	2,296	,182
	Error	38,185,111	6	6364,185 ^b		
Treatment * Month * Site	Hypothesis	38,185,111	6	6,364,185	,959	,466
	Error	238,846,667	36	6634,630 ^c		

a. MS(Treatment * Site)

b. MS(Treatment * Month * Site)

c. MS(Error)

Tests of Between-Subjects Effects

Dependent Variable: Total Species (S)

Source		Type III Sum of Squares	df	Mean Square	F	Sig.
Treatment	Hypothesis	8,037	2	4,019	,063	,940
	Error	382,889	6	63,815 ^a		
Treatment * Site	Hypothesis	382,889	6	63,815	2,056	,201
	Error	186,222	6	31,037 ^b		
Month	Hypothesis	10,667	1	10,667	,344	,579
	Error	186,222	6	31,037 ^b		
Treatment * Month	Hypothesis	84,778	2	42,389	1,366	,324
	Error	186,222	6	31,037 ^b		
Treatment * Month * Site	Hypothesis	186,222	6	31,037	2,103	,077
	Error	531,333	36	14,759 ^c		

a. MS(Treatment * Site)

b. MS(Treatment * Month * Site)

c. MS(Error)

Continuation of Table 3.

Tests of Between-Subjects Effects

Dependent Variable: Shannon-Wiener Transformed with Hill (N1)

Source		Type III Sum of Squares	df	Mean Square	F	Sig.
Treatment	Hypothesis	44,593	2	22,296	1,282	,344
	Error	104,333	6	17,389 ^a		
Treatment * Site	Hypothesis	104,333	6	17,389	1,704	,267
	Error	61,222	6	10,204 ^b		
Month	Hypothesis	8,963	1	8,963	,878	,385
	Error	61,222	6	10,204 ^b		
Treatment * Month	Hypothesis	13,481	2	6,741	,661	,550
	Error	61,222	6	10,204 ^b		
Treatment * Month * Site	Hypothesis	61,222	6	10,204	1,625	,169
	Error	226,000	36	6,278 ^c		

a. MS(Treatment * Site)

b. MS(Treatment * Month * Site)

c. MS(Error)

Tests of Between-Subjects Effects

Dependent Variable: Simpson Transformed with Hill (N1)

Source		Type III Sum of Squares	df	Mean Square	F	Sig.
Treatment	Hypothesis	12,704	2	6,352	,514	,622
	Error	74,111	6	12,352 ^a		
Treatment * Site	Hypothesis	74,111	6	12,352	1,434	,336
	Error	51,667	6	8,611 ^b		
Month	Hypothesis	6,000	1	6,000	,697	,436
	Error	51,667	6	8,611 ^b		
Treatment * Month	Hypothesis	9,000	2	4,500	,523	,618
	Error	51,667	6	8,611 ^b		
Treatment * Month * Site	Hypothesis	51,667	6	8,611	1,587	,179
	Error	195,333	36	5,426 ^c		

a. MS(Treatment * Site)

b. MS(Treatment * Month * Site)

c. MS(Error)

Continuation of table 3.

Tests of Between-Subjects Effects

Dependent Variable: Density

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Treatment Hypothesis	5,251,290,778	2	2,625,645,389	1,141	,380
Treatment Error	13,807,143,222	6	2301190,537 ^a		
Treatment Hypothesis	13,807,143,222	6	2,301,190,537	2,928	,108
* Site Error	4,715,510,111	6	785918,352 ^b		
Month Hypothesis	3,122,892,519	1	3,122,892,519	3,974	,093
Month Error	4,715,510,111	6	785918,352 ^b		
Treatment Hypothesis	3,608,007,370	2	1,804,003,685	2,295	,182
* Month Error	4,715,510,111	6	785918,352 ^b		
Treatment Hypothesis	4,715,510,111	6	785,918,352	,959	,466
* Month * Site Error	29,488,471,333	36	819124,204 ^c		

a. MS(Treatment * Site)

b. MS(Treatment * Month * Site)

c. MS(Error)

Tests of Between-Subjects Effects

Dependent Variable: Abundance

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Treatment Hypothesis	5,752,778	2	2,876,389	,289	,759
Treatment Error	59,615,222	6	9935,870 ^a		
Treatment Hypothesis	59,615,222	6	9,935,870	,862	,569
* Site Error	69,156,556	6	11526,093 ^b		
Month Hypothesis	39,744,907	1	39,744,907	3,448	,113
Month Error	69,156,556	6	11526,093 ^b		
Treatment Hypothesis	42,296,037	2	21,148,019	1,835	,239
* Month Error	69,156,556	6	11526,093 ^b		
Treatment Hypothesis	69,156,556	6	11,526,093	3,198	,013
* Month * Site Error	129,757,333	36	3604,370 ^c		

a. MS(Treatment * Site)

b. MS(Treatment * Month * Site)

c. MS(Error)

Appendix 2. P values of One way ANOVA performed with environmental data. Groups are streams

classified under the same treatment in the Mashpi basin, Ecuador.

		Sum of Squares	df	Mean Square	F	Sig.
Temperature	Between Groups	,282	2	,141	13,368	,006
	Within Groups	,063	6	,011		
	Total	,346	8			
Conductivity	Between Groups	114,667	2	57,334	,306	,748
	Within Groups	1125,833	6	187,639		
	Total	1240,501	8			
Oxygen	Between Groups	,583	2	,292	2,811	,138
	Within Groups	,623	6	,104		
	Total	1,206	8			
pH	Between Groups	,244	2	,122	6,973	,027
	Within Groups	,105	6	,017		
	Total	,349	8			
D16	Between Groups	,000	2	,000	.	.
	Within Groups	,000	6	,000		
	Total	,000	8			
D50	Between Groups	15,167	2	7,583	,498	,631
	Within Groups	91,333	6	15,222		
	Total	106,500	8			
D84	Between Groups	37,389	2	18,694	,124	,885
	Within Groups	901,833	6	150,306		
	Total	939,222	8			
Sg	Between Groups	,049	2	,024	,214	,813
	Within Groups	,684	6	,114		
	Total	,733	8			
Discharge	Between Groups	,001	2	,001	4,455	,065
	Within Groups	,001	6	,000		
	Total	,002	8			
CPOM	Between Groups	55,710	2	27,855	2,874	,133
	Within Groups	58,153	6	9,692		
	Total	113,863	8			
Nitrates	Between Groups	,014	2	,007	4,167	,073
	Within Groups	,010	60.748	,002		
	Total	,024	8			
Phosphates	Between Groups	1,241	2	,620	,844	,476
	Within Groups	4,413	6	,736		
	Total	5,654	8			

Appendix 3. Test for normal distribution of environmental variables using Kolmogorov Smirnov and Lilliefors tests, performed in Statistica. The phosphate concentration was the only variable with an abnormal distribution on the Kolmogorov test.

Tests of Normality						
	N	max D	K-S – p	Lilliefors – p	W	p
Site	9	0.100729	p > .20	p > .20	0.972288	0.913561
Temperature°C	9	0.248276	p > .20	p < .15	0.879765	0.156127
Conductivity	9	0.253364	p > .20	p < .10	0.917888	0.375043
Oxygen(mg/L)	9	0.17888	p > .20	p > .20	0.944806	0.633297
pH	9	0.297502	p > .20	p < .05	0.76854	0.008886
Sustrate	9	0.178716	p > .20	p > .20	0.928302	0.465343
Discharge	9	0.22814	p > .20	p < .20	0.864841	0.1082
CPOM	9	0.349587	p < .20	p < .01	0.723002	0.002632
Nitrates	9	0.35834	p < .20	p < .01	0.785024	0.013757
PhosphatesConc890	9	0.464774	p < .05	p < .01	0.468869	0.000003

Appendix 4. Mean values comprise of the different months, the environmental variables were measured at the 9 sites

Site	Mean Temperature	Mean Conductivity	Oxygen(mg/l)	Mean pH	D50	D84	SG	Discharge (cm ³ /s)	CPOM(g/m ²)	Nitrates(mg/l)	Phosphates(mg/l)
Boshungo	21.20	20.20	7.40	7.38	13	32	2.13	0.01	6.73	0.40	0.11
Chakra	21.35	46.05	7.50	7.78	18	61	2.92	0.02	9.85	0.40	0.26
MalTrib	21.35	45.05	6.93	7.74	26	57	2.85	0.01	16.70	0.45	0.19
Ines	21.50	33.40	7.26	7.74	23	63	3.02	0.04	3.90	0.30	0.06
Mashungo	21.40	30.80	7.01	7.77	20	46	2.57	0.01	6.35	0.40	0.13
Pamb	21.70	64.30	7.60	7.86	19	51	2.70	0.04	6.50	0.30	0.23
Maspest1	21.75	44.60	6.85	7.39	19	48	2.55	0.00	5.50	0.40	0.07
Maspest2	21.75	45.70	7.04	7.39	18	61	2.92	0.00	7.20	0.40	0.12
Taipest	21.70	46.75	6.34	7.39	16	38	2.30	0.01	5.55	0.45	2.66

Appendix 5. Community raw metrics of all nine streams studied. Rivers were classified under treatment type. S (Diversity), N (Abundance), N1 and N2 (log transformed abundances), Density (Surber) and Relative abundance (Kicks).

Treatment	Code	Month	S	N	N1	N2	Dens(Surber)	RelativeAbund(kick)
Forest	Boshungo	February	20	124	11	7	1381	154
Forest	Chakra	February	21	138	9	5	1530	95
Forest	Maltrib	February	21	156	13	9	1737	145
Forest	Boshungo	May	18	54	12	9	604	59
Forest	Chakra	May	22	235	9	6	2615	229
Forest	Maltrib	May	23	216	10	6	2404	140
Farm	Ines	February	26	126	13	8	1400	83
Farm	Pamb	February	21	114	12	8	1267	100
Farm	Mashung	February	20	114	9	6	1267	162
Farm	Ines	May	23	71	16	11	785	86
Farm	Pamb	May	18	82	9	6	911	89
Farm	Mashung	May	20	185	11	7	2059	224
Monoculture	Taipest	February	15	121	8	6	1344	107
Monoculture	Maspest1	February	27	216	10	5	2400	70
Monoculture	Maspest2	February	13	56	8	6	619	71
Monoculture	Taipest	May	25	214	13	9	2374	135
Monoculture	Maspest1	May	23	311	8	5	3456	265
Monoculture	Maspest2	May	20	186	11	8	2067	248